Table III. Effect of Sesamex on in Vivo Metabolism of Diflubenzuron in Larvae of S-NAIDM, R-OMS-12, and R-diflubenzuron Strains of Housefly[a]

	external fraction						internal fraction							
	diflubenzuron metabolites						diflubenzuron metabolites							
	organo-soluble		polar[b]		unmetabolized diflubenzuron		organo-soluble		polar[b]		unmetabolized diflubenzuron		unextractable radiolabel	
	alone	+sesamex	alone	+sesamex	alone	+sesamex	alone	+sesamex	alone	+sesamex	alone	+sesamex	alone	+sesamex
					[*aniline*-^{14}C]Diflubenzuron									
S-NAIDM	13.3	7.8	17.9	2.6	25.8	38.3	5.7	3.1	9.2	1.2	18.2	31.3	9.7	16.6
R-OMS-12	8.8	3.4	24.8	6.1	41.4	51.4	3.3	2.8	18.7	2.2	0.6	28.9	2.9	7.1
R-diflubenzuron	10.9	5.3	33.6	16.2	41.0	51.8	1.0	4.6	8.2	6.7	1.4	9.7	3.2	5.8
					[*benzoyl*-^{3}H]Diflubenzuron									
S-NAIDM	15.9	1.0	12.5	1.1	35.5	47.7	2.3	0.5	4.5	0.7	21.8	37.9	9.3	11.2
R-OMS-12	11.9	7.1	21.6	6.3	44.9	57.7	2.3	0.8	12.5	1.8	0.4	13.4	7.7	12.8
R-diflubenzuron	10.6	3.8	34.2	14.2	38.0	59.7	7.1	0.2	5.0	1.3	0.9	12.6	4.2	8.3

[a] Percent of the total radioactivity recovered. [b] Polar metabolites constitute hydroxylated diflubenzuron, methanol-soluble metabolites, and water-soluble conjugates.

mechanism of diflubenzuron resistance, then the levels of synergism (SR ratios) should correspond to the levels of diflubenzuron resistance (RR ratios) exhibited by the R strains. This was not found to be entirely the case. The log dose–probit mortality lines for the S-NAIDM (without synergist), R-OMS-12, and R-diflubenzuron strains (with synergist) (Figure 1) demonstrate that resistance to diflubenzuron in the R strains is not completely abolished by MFO inhibitors. This suggests that other factors such as esterases, glutathione-dependent transferases, slower cuticular penetration, or decreased effectiveness at the site of action may also be contributing to diflubenzuron resistance.

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Oxidative Cleavage of the Disulfide Bond of Cystine by Allyl Isothiocyanate

Interaction of isothiocyanate with L-cystine under mild conditions was studied in detail. Electrophilic attack of allyl isothiocyanate to cystine resulted in scission of the sulfide bond to give two thiazoline derivatives, 2-amino- (**3**) and 2-(allylamino)-2-thiazoline-4-carboxylic acid (**4**), and their formation mechanisms were proposed.

It is well-known that many kinds of alkyl isothiocyanates are formed from glucosinolates by the action of myrosinase in crushed tissues of *Brassica* species such as rapeseed, radish root, etc., and they usually show strong pungent tastes. Most of isothiocyanates are not so stable and gradually decomposed to unpungent products in the presence of water (Gmelin et al., 1960; Gmelin and Virtanen, 1962; Kawakishi and Muramatsu, 1966; Kawakishi et al., 1967; Kawakishi and Namiki, 1969). Those are strong electrophilic reagents and easily react with some nucleophiles such as amines, water, and alcohols under mild condition to give the corresponding adducts. On the other hand, synthetic phenyl isothiocyanate has been shown to readily react with the N-terminal amino acid of protein to afford phenyl thiocarbamoyl protein and subsequently phenylthiohydantoin of the amino acid on dehydration (Edman, 1949, 1950). Therefore, isothiocyanates formed in the crushed *Brassica* tissues may arise from some chemical modification of tissue proteins under physiological condition.

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The present work was undertaken to study the interaction of allyl isothiocyanate produced from sinigrin, a most widely distributed glucosinolate in *Cruciferae* family, with certain amino acids and proteins under mild conditions. Here, we have reported the oxidative cleavage of the disulfide bond of L-cystine with allyl isothiocyanate.

EXPERIMENTAL SECTION

Reaction Mixture. The aqueous suspension (500 mL) composed of L-cystine (2 mmol) and freshly distilled allyl isothiocyanate (50 mmol) was adjusted in pH 6.0 and heated for 2 h at 80 °C with stirring under a nitrogen atmosphere.

DTNB Method and TLC. The reaction process was followed by the formation of SH or SOH compounds as intermediates with the DTNB method (Ellman, 1959; Janatova et al., 1968). Decomposition of cystine during the reaction was examined by TLC using Merck DC-Plastikfolien cellulose plates with a solvent system of 1-BuOH–AcOH–H_2O, 4:1:5 v/v, and the color development with 0.2% ninhydrin.

HPLC. The reaction mixture was extracted with ether, and the aqueous solution was concentrated to dryness under reduced pressure. The pasty dry matter was dissolved in methanol–water (1:2.5 v/v, 5 mL) and submitted to HPLC (Waters, ALC/GPC 202) equipped with a Bondapak C_{18} Polasil B column (0.8 × 120 cm). Elution was carried out with methanol–water (1:2.5 v/v, 5 mL/min). Repeated HPLC was performed by the use of a 10 ODS Develosil column (0.8 × 25 cm) with methanol–water (1:9 v/v, 3 mL/min).

NMR Spectra. NMR spectra were obtained with a JEOL model JNM FX-100 spectrometer.

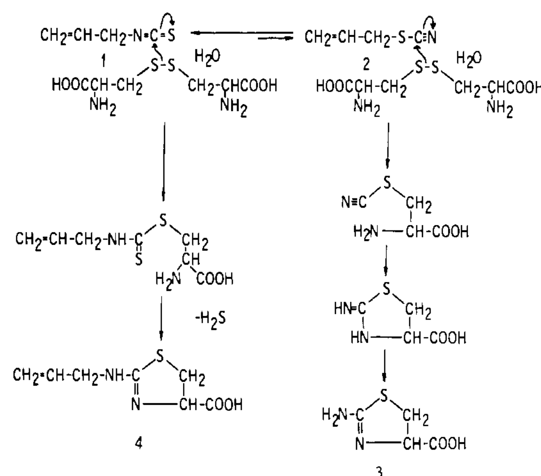
Mass Spectra. A JEOL Model JMS D-100 mass spectrometer was used in the direct inlet mode under the following conditions: ionizing voltage, 75 eV; ionizing current, 300 μA; ion source temperature, 250 °C.

RESULTS AND DISCUSSION

The reaction of allyl isothiocyanate with cystine proceeded rapidly at 80 °C, and the color development with DTNB reached to its maximum within 1 h and cystine disappeared in the reaction mixture after 2 h. In HPLC of the pasty dry matter (405 mg) using the Bondapak C_{18} column, two large peaks, 1 and 2, were preparatively isolated, and the product **2** (P_2) was obtained from the peak 2 in a crystalline form (64 mg). The peak 1 (dry matter, 102 mg) was shown to be a mixture of several products by TLC, and the concentrate of peak 1 was separated to four peaks by the repeated HPLC using the 10 ODS column, in which the peak 1-1 gave crystalline $P_{1\text{-}1}$ (15 mg): mp 200–205 °C dec (from ethanol–water); IR ν_{max} (KBr) 1720, 1640, and 1200 cm^{-1}; MS m/e (rel intensity) 146 (M^+, 3), 128 ($M^+ - H_2O$, 2), and 101 ($M^+ - CO_2H$, 100); ^1H NMR (D_2O + DCl vs. acetone) (thiazoline ring H) δ 3.66 (1 H, dd, J = 11.2, 4.7 Hz), 3.86 (1 H, dd, J = 11.2, 8.4 Hz), and 4.90 (1 H, dd, J. = 8.4, 4.7 Hz). Anal. Found: C, 32.40; H, 4.22; N, 18.48. Calcd for $C_4H_6N_2O_2S$: C, 32.88; H, 4.14; N, 19.18. These data suggested 2-amino-2-thiazoline-4-carboxylic acid (**3**) (Schöberi et al., 1951; Behringer and Zillikens, 1951) for the structure of $P_{1\text{-}1}$, which agreed well with the synthetic product from L-cystine and potassium cyanide (Jacobson et al., 1973).

Data for the product P_2 were as follows: mp 186 °C (from methanol); IR ν_{max} (KBr) 1600, 1390, 995, and 920 cm^{-1}; MS m/e (rel intensity) 186 (M^+, 14), 141 ($M^+ - CO_2H$, 100), and 114 ($M^+ - CO_2H - CH{=}CH_2$, 65); ^1H NMR (CD_3OD vs. Me_4Si) (thiazoline ring H) δ 3.70 (1 H,

Scheme I



dd, J = 11.3, 5.4 Hz), 3.86 (1 H, dd, J = 11.3, 8.1 Hz), 4.63 (1 H, dd, J = 8.1, 5.4 Hz); ^1H NMR (CD_3OD vs. Me_4Si) (allyl group H) δ 4.05 (2 H, d, J = 5.4 Hz), 5.28 (1 H, m), 5.33 (1 H, m), and 5.93 (1 H, m). Anal. Found: C, 45.32; H, 5.49; N, 15.00. Calcd for $C_7H_{10}N_2O_2S$: C, 45.15; H, 5.41; N, 15.05. From these results, the structure of P_2 was easily estimated as 2-(allylamino)-2-thiazoline-4-carboxylic acid (**4**).

Since both products were also formed under the lower temperature at 40–60 °C at pH 6.0, the formation of **3** from **4** through the elimination of the allyl group was improbable under such mild conditions. Therefore, the formation of two products may be considered as shown in Scheme I. The electrophilic attack of allyl isothiocyanate (**1**) to the disulfide bond of cystine may cause oxidative fission to give dithiocarbamate which promptly cyclizes to the thiazoline derivative (**4**) through a release of hydrogen sulfide. On the other hand, **1** may partially isomerize to thiocyanate in its aqueous solution as is the case for benzyl isothiocyanate (Saarivirta and Virtanen, 1963), and the resulting allyl thiocyanate (**2**) may also react with cystine to afford β-thiocyanoalanine which will easily cyclizes to iminothiazolidine and subsequently isomerizes to 2-amino-2-thiazoline-4-carboxylic acid (**3**). If the cleavage of cystine proceeds as shown in the proposed scheme, the other half moiety of cystine will be transformed into a cystein-*S*-yl cation, but its derivative is not yet identified at the present time. This interaction suggests the possibility of the oxidative cleavage of the disulfide bond of the cystine moiety in some proteins by the action of isothiocyanate in crushed *Cruciferae* tissues.

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Albumin Proteins of Eight Edible Grain Legume Species: Electrophoretic Patterns and Amino Acid Composition

The albumin protein (AP) content of eight edible grain legume species varied from 8.1 to 14.1% of the meal protein. The AP contained from 9.5 to 12.4% nitrogen, 0.09 to 1.72% phosphorus, and 0.2 to 1.8% starch. Although there were differences in the amino acid composition of the species, the AP contained more tryptophan, lysine, threonine, valine, and methionine but less arginine, leucine, and phenylalanine than the globulin proteins isolated from the same legume species. The AP of mung beans, lentils, faba beans, vetch, dry beans, and peas resolved into 20–25 bands and the AP of lathyrus and chickpeas into 19 bands on sodium dodecyl sulfate–polyacrylamide gel electrophoresis with molecular weights estimated mostly between 10 000 and 100 000.

The albumin proteins (AP) are minor and the globulin proteins (GP) major proteins of legume seeds. The GP of a number of legume species have been investigated (Derbyshire et al., 1976; Barker et al., 1976; Stockman et al., 1976; Krishna et al., 1977, 1979; Utsumi and Mori, 1980; Mutschler et al., 1980). In contrast, only a few studies have been conducted on the AP of legumes (Fox et al., 1964; Grant et al., 1976; Sefa-Dedeh and Stanley, 1979; Murray, 1979). Unlike the GP, which are storage proteins, the AP are mostly enzymic or nonstorage proteins. However, recent work (Murray, 1979) showed that AP of pea cotyledons were degraded during germination and thus behaved like the GP or storage proteins.

The AP of peas, faba beans, and chickpeas were richer in sulfur amino acids and lysine than the GP (Jackson et al., 1969; Bajaj et al., 1971) and may be used to improve the methionine–cystine levels of edible grain legumes. However, this view is not shared by Boulter et al. (1973). The functional properties of protein isolates prepared from AP of peas have been reported (Grant et al., 1976).

Fox et al. (1964) reported the electrophoretic patterns of AP isolated from 17 species of legumes (mostly nonedible grain legumes). Nevertheless, most studies on AP have been conducted with peas. Thus, there is a paucity of information on the AP of other legumes, particularly the edible grain legumes which are being used increasingly in human foods. The present study reports on the level, amino acid composition, and electrophoretic patterns of AP isolated from eight species of common edible grain legumes.

MATERIALS AND METHODS

The eight species of edible grain legumes used in the study were the following: chickpeas (*Cicer arietinum* L.), line PI 239859; dry beans (*Phaseolus vulgaris*), cv. Saxa; faba beans (*Vicia faba* L. ssp. minor), cv. Diana; lentils (*Lens culinaris* Medic), type Common Chilean; peas (*Pisum sativum* L.), cv. Trapper; vetch (*Vicia sativa* L.), cv. Alger Somm; mung bean [*Vigna radiata* (L.) Wilezek], line UM MBI; Lathyrus (*Lathyrus sativus* L.), line 2R27. The legumes were grown by Dr. A. E. Slinkard of this department at the University of Saskatchewan experimental plots, Saskatoon. The legume samples were cleaned and ground in a Udy cyclone mill to pass a 1.0-mm screen, and the meal was stored at 5 °C.

The extraction and separation of AP and GP was largely based on the method of Murray (1979). Each meal was vigorously shaken at 5 °C for 10 min with 5.0% potassium sulfate (pH 7.0) in a Udy multiple shaker. The meal to solvent ratio was 1 to 20. The extract was centrifuged at 12000*g* (5 °C) for 20 min; the residue was reextracted once more as described above. The supernatants from the two extractions were combined. An aliquot was taken for total nitrogen determination to calculate salt-soluble nitrogen and the rest of the extract dialyzed at 5 °C for 36 h against several changes of deionized distilled water. The dialyzed extract was centrifuged at 12000*g* for 20 min to obtain water-soluble (AP) and water-insoluble (GP) fractions, which were freeze-dried.

The freeze-dried AP was analyzed in duplicate for total nitrogen, phosphorus, and starch, and the AP and GP were analyzed for amino acids, except methionine, as described previously (Bhatty and Slinkard, 1979). The methionine content of AP and GP was determined by gas–liquid chromatography (Finlayson and MacKenzie, 1976).

Sodium dodecyl sulfate ($NaDodSO_4$)–polyacrylamide gel electrophoresis of AP was conducted according to Weber et al. (1968) at an acrylamide concentration of 10%. Molecular weight of the proteins was estimated from a low molecular weight standard (M_r range from 14 300 to 94 000; Pharmacia, Montreal) electrophoresed under identical conditions. Mobilities of the electrophoretic bands were measured relative to that of the tracking dye. The relationship between log molecular weight and relative mobility was linear.

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